APPENDIX C: Multiple Active Ingredient Product Analysis

The Agency does not routinely include, in its risk assessments, an evaluation of mixtures of active ingredients, either those mixtures of multiple active ingredients in product formulations or those in the applicator's tank. In the case of the product formulations of active ingredients (that is, a registered product containing more than one active ingredient), each active ingredient is subject to an individual risk assessment for regulatory decision regarding the active ingredient on a particular use site. If effects data are available for a formulated product containing more than one active ingredient, they may be used qualitatively or quantitatively^{1,2}.

Acute oral toxicity data (i.e., LD50 values) from mammalian studies for formulated products that contain atrazine and one or more additional active ingredients are summarized below.

Currently, the Agency's guidance for assessing the potential risk of chemical mixtures is limited to human health applications (USEPA, 2000). However, the guidance includes principles for evaluating mixtures to assess potential interactive effects that are generally applicable. Consistent with EPA's Overview Document (USEPA 2004), the Agency's mixture guidance (USEPA 2000) discusses limitations in quantifying the risk of specified mixtures when there is differential degradation, transport and fate of chemical components following environmental release or application. The LD50 values are potentially useful only to the extent that a wild mammal would consume plants or animals immediately after these dietary items were directly sprayed by the product. Increasing time post application, the differential rates of degradation, transport, etc. for the active ingredients in the formulation only permit a qualitative discussion of potential acute risk (USEPA 2004).

As discussed in USEPA (2000) a quantitative component-based evaluation of mixture toxicity requires data of appropriate quality for each component of a mixture. In this mixture evaluation LD50s, with associated 95% confidence intervals, are needed for the formulated product. The same quality of data is also required for each component of the mixture. Given that many of the formulated products do not have LD50 values of the required quality and since LD50 values are not available for all the components of these formulations a quantitative analysis of potential interactive effects is not possible.

While a quantitative evaluation of the data is not possible with currently accepted scientific methods, as a screening tool, a qualitative analysis can be used to indicate if formulated products exhibit interactive effects (e.g., synergism or antagonism).

In the case of atrazine, a qualitative examination of the trends in LD50 values, with the associated confidence intervals, across the range of percent active ingredient, show no

¹ Overview of the Ecological Risk Assessment Process in the Office of Pesticide Programs, Environmental Protection Agency (January 2004) (Overview Document).

² Memorandum to Office of Prevention, Pesticides and Toxic Substance, US EPA conveying an evaluation by the U.S. Fish and Wildlife Service and National Marine Fisheries Service of an approach to assessing the ecological risks of pesticide products (January 2004).

discernable trends in potency that would suggest synergistic (i.e., more than additive) or antagonistic (i.e., less than additive) interactions.

When the product LD50 values (with associated confidence intervals) were available for each active ingredient in a product, the predicted LD50s for the formulated products were estimated by considering the proportion and potency of each active ingredient in the mixture. The formula[3] is presented below, where r equals the relative proportion of each active ingredient (ai) in the formulated product (f).

Estimated LD50_(f)=
$$[r_{ai1}/LD50_{(ai1)} + r_{ai2}/LD50_{(ai2)}]^{-1}$$

The estimated LD50 formula assumes no synergistic or antagonistic interactions. Estimated LD50 values above or below the LD50 confidence intervals for the formulated product could suggest an interactive effect. In all six cases, the estimated LD50s fell either within the confidence intervals for the formulated products. These results provide additional confidence that synergistic interactions are unlikely for the formulated products examined.

Based on these evaluations of the best available data and the Agency's existing guidance it is reasonable to conclude that these formulations are reflecting an independent additive toxicity response and not an interactive effect. Given that the active and inert ingredients would not be expected to have similar mechanisms of action, metabolites or toxicokinetic behavior it is also reasonable to conclude that an assumption of dose-addition would be inappropriate. Consequently, an assessment of atrazine's potential effect on the CRLF when it is co-formulated with other active ingredients can be based on the toxicity of atrazine.

Review of Open Literature Studies on Multiple Active Ingredient Products

Hayes et al. (2006) assessed the effect of Bicep II Magnum (reported as 33.3% atrazine, 0.7% atrazine-related products, 26.1% TGAI of S-metolachlor, and 40.2% inert ingredients) to mortality, growth and development, gonadal development, thymus histology, and disease rates (i.e., immune function) in larval leopard frogs (*R. pipiens*). Based on the Agency's review of the Hayes et al. (2006) study, there are a number of uncertainties which confound the ability to interpret the study results. The results of this study, including a discussion of associated uncertainties, are summarized in Section A.2.4d and Table A.16 of Appendix A.

In the 2008 Ecotox search seven studies were rejected from the public version of Ecotox because mixtures were tested. These data include the following:

- Douglas, W. S., McIntosh, A., and Clausen, J. C. Toxicity of Sediments Containing Atrazine and Carbofuran to Larvae of the Midge Chironomus tentans. Environ. Toxicol. Chem. 12, 847-853 (OECDG Data File). 93.
- 2. Faust, M., Altenburger, R., Boedeker, W., and Grimme, L. H. Additive Effects of Herbicide Combinations on Aquatic Non-Target Organisms. Sci.Total Environ. 134 (Suppl.2), 941-952. 93.
- 3. Fitzgerald, C. H., Richards, R. F., Selden, C. W., and May, J. T. Three Year Effects of Herbaceous Weed

^{[&}lt;sup>3</sup>] Methods described in Tabashnik, BE, Evaluation of Synergism among *Bacillus thuringiensis* Toxins, Appl Environ Microbiol. 1992 Oct;58(10):3343-6.

Control in a Sycamore Plantation. Weed Sci. 23(1), 32-35. 75.

- 4. Hoagland, K. D., Drenner, R. W., Smith, J. D., and Cross, D. R. Freshwater Community Responses to Mixtures of Agricultural Pesticides: Effects of Atrazine and Bifenthrin. Environ. Toxicol. Chem. 12, 627-637 (OECDG Data File). 93.
- 5. Roberts, S., Vasseur, P., and Dive, D. Combined Effects Between Atrazine, Copper and pH, on Target and Non Target Species. Water Res. 24(4), 485-491. 90. W
- 6. Roslycky, E. B. Response of Soil Microbiota to Selected Herbicide Treatments. Can.J.Microbiol. 23(4), 426-433.77.
- 7. Sauls, B. T. Pesticide Risk Mitigation of Avian Wildlife in Iowa Agroecosystems. Ph.D.Thesis, Texas Tech Univ., Lubbock, TX, 104 p. 99.

<u>Pesticide Products Formulated with Atrazine and Other Pesticide Active Ingredients</u>

$\underline{ATRAZINE\ PRODUCTS}^{\quad i\quad ii}$

PRODUCT NAME	EPA Reg.No.	% Atrazine	PRODUCT		ADJUSTED FOR ACTIVE INGREDIENT	
			LD 50 (mg/kg)	CI (mg/kg)	LD50 (mg/kg)	CI (mg/kg)
BICEP II MAGNUM						
HERBICIDE	000100-00817	33	4824	No Data	1592	N/A
BICEP LITE II MAGNUM HERBICIDE	000100-00827	28.1	4824	No Data	1356	N/A
BICEP MAGNUM	000100-00886	33.7	6044	4480-7487	2037	1510-2523
LUMAX SELECTIVE HERBICIDE	000100-01152	11	2865	No Data	315	N/A
			>2000			
EXPERT HERBICIDE	000100-01161	22.9	&<5000	No Data	N/A	N/A
BRAWN HERBICIDE	000100-01165	33	3271	2755-3882	1079	909-1281
LEXAR HERBICIDE	000100-01201	19	4144	1485- 20000	787	282-3800
BICEP LITE II MAGNUM MANUFACTURING USE						
PRODUCT	000100-01213	28.13	No Data	No Data	N/A	N/A
BICEP II MAGNUM MANUFACTURING USE						
PRODUCT	000100-01214	33.7	3271	2755-3882	1102	928-1308
BUCTRIL + ATRAZINE HERBICIDE	000264-00477	21.62	3371	2382-4013	728	515-868
LIBERTY ATZ HERBICIDE	000264-00668	31.75	2119	No Data	673	N/A
BASIS GOLD HERBICIDE	000352-00585	86.78	2245	1656-3044	1948	1437-2642
DPX-MX670 MT	000352-00600	28.4	No Data	No Data	N/A	N/A
DUPONT STEADFAST ATZ HERBICIDE	000352-00619	85.3	2245	1656-3044	1915	1413-2597
DUPONT CINCH ATZ LITE HERBICIDE	000352-00623	28.1	3621	No Data	1018	N/A
DUPONT CINCH ATZ HERBICIDE	000352-00624	33	3271	2755-3882	1079	909-1281
DUPONT BREAKFREE ATZ LITE	000352-00723	16.3	No Data	No Data	N/A	N/A
DUPONT DPX-QDN33 HERBICIDE	000352-00724	24.4	No Data	No Data	N/A	N/A
LARIAT HERBICIDE	000524-00329	16	4400	No Data	704	N/A
BULLET HERBICIDE	000524-00418	14.5	>7500	N/A	N/A	N/A
HARNESS XTRA HERBICIDE	000524-00480	18.3	1249	1043-1495	229	191-274
HARNESS XTRA 5.6L HERBICIDE PREMIX FOR CORN	000524-00485	26.9	1338	1153-1552	360	310-417
MON 58442 HERBICIDE	000524-00497	16.2	3105	2563-3761	503	415-609
MON 78088 HERBICIDE	000524-00509	20.9	3979	3419-4632	832	715-968
MON 58494 HERBICIDE	000524-00511	14.5	>5000	N/A	N/A	N/A

PRODUCT NAME	EPA Reg.No.	% Atrazine	PRODUCT		ADJUSTED FOR ACTIVE INGREDIENT	
			LD 50 (mg/kg)	CI (mg/kg)	LD50 (mg/kg)	CI (mg/kg)
BALLISTIC	000534-00098	30	No Data	No Data	N/A	N/A
SOUTHERNMAX	000538-00301	1.067	>5000	N/A	N/A	N/A
SOUTHERNMAX PRO	000538-00302	1.067	>5000	N/A	N/A	N/A
CHARGER MAX ATZ	001381-00199	33.7	No Data	No Data	N/A	N/A
CHARGER MAX ATZ LITE	001381-00199	28.1	No Data	No Data	N/A	N/A
MARKSMAN HERBICIDE	007969-00136	22.23	5897	4797-7250	1311	1066-1612
GUARDSMAN MAX	007909-00130	22.23	>500 &	4/9/-1230	1311	1000-1012
HERBICIDE	007969-00192	35.3	<2000 &	N/A	N/A	N/A
G-MAX LITE	007969-00200	29.5	>500 & <2000	N/A	N/A	N/A
DREXEL SIMAZAT 4L HERBICIDE	019713-00171	21.03	3600	2800-5000	757	589-1051
DREXEL ACETOCHLOR PLUS ATRAZINE	019713-00513	16.6	No Data	No Data	N/A	N/A
FLUS ATRAZINE	019/13-00313	10.0	No Data	No Data	IN/A	IN/A
DREXEL TRIZMET II	019713-00547	33.1	>2000	N/A	N/A	N/A
DREXEL SIMAZAT 90DF	019713-00553	44.18	>2000	N/A	N/A	N/A
TREMOR AT	033270-00013	24.4	>2000	N/A	N/A	N/A
TREMOR AT LITE STRATOS DICAMBA+ATRAZINE AGRICULTURAL HERBICIDE	033270-00014	16.3 21.92	No Data 5897	No Data 4797-7250	N/A 1293	N/A 1052-1589
SHOTGUN FLOWABLE	033038-00010	21.92	3897	4/9/-/230	1295	1032-1369
HERBICIDE	034704-00728	24.24	>5000	N/A	N/A	N/A
RIFLE PLUS HERBICIDE	034704-00860	22.23	No Data	No Data	N/A	N/A
BROZINE	034704-00892	21.62	No Data	No Data	N/A	N/A
CADENCE ATZ HERBICIDE CADENCE LITE ATZ	034704-00950	24.4	No Data	No Data	N/A	N/A
HERBICIDE	034704-00952	16.3	No Data	No Data	N/A	N/A
DICAMBAZINE	042750-00041	22.23	>5050	N/A	N/A	N/A
BROX-AT HERBICIDE	042750-00050	21.62	3981	3319-4775	861	718-1032
ACETOCHLOR 4.3 + ATZ 1.7	042750-00106	18.3	2599	1240-5000	476	227-915
ACETOCHLOR 3.1 + ATZ 2.5	042750-00108	26.9	1338	No Data	360	N/A
			>1000 &			
PROMPT 5L HERBICIDE	051036-00363	25	<2000	No Data	N/A	N/A
LADDOK 5L HERBICIDE	051036-00415	24.4	3840	2700-6410	936	659-1564
DICAMBA-ZINE	053883-00143	21.92	No Data	No Data	N/A	N/A
VOLLEY ATZ LITE TENKOZ HERBICIDE	055467-00006	16.3	3030	No Data	494	N/A

			PRODUCT		ADJUSTED FOR ACTIVE INGREDIENT	
PRODUCT NAME	EPA Reg.No.	% Atrazine	LD 50 (mg/kg)	CI (mg/kg)	LD50 (mg/kg)	CI (mg/kg)
VOLLEY ATZ TENKOZ	055467 00007	24.4	2020	N. D.	720	NT/A
HERBICIDE	055467-00007	24.4	3030	No Data	739	N/A
STALWART EXTRA	060063-00023	33	>2000	N/A	N/A	N/A
KEYSTONE HERBICIDE	062719-00368	24.4	2242	1272-3952	547	310-964
FULTIME SELECTIVE HERBICIDE	062719-00371	16.6	>5000	N/A	N/A	N/A
KEYSTONE LA HERBICIDE	062719-00479	16.3	>2000 & <5000	N/A	N/A	N/A
BROMOXYNIL + ATRAZINE HERBICIDE	066222-00108	21.62	1140	380-2950	246	82-638
TRIANGLE HERBICIDE	066222-00131	28.6	>2000	N/A	N/A	N/A
PARALLEL PLUS	066222-00132	30	>2000	N/A	N/A	N/A
BANVEL + ATRAZINE	066330-00286	22.23	5897	4797-7250	1311	1066-1612
BROMOX + ATRAZINE	071368-00068	21.62	3093	No Data	669	N/A

References

- Hayes, T.B., P. Case, S. Chui, D. Chung, C. Haeffele, K. Haston, M. Lee, V.P. Mai, Y. Marjuoa, J. Parker, and M. Tsui. 2006. Pesticide Mixtures, Endocrine Disruption, and Amphibian Declines: Are We Understanding the Impact? Env. Health Persp. 114(1) p. 40-50.
- U.S. Environmental Protection Agency (U.S. EPA). 2000. Supplementary guidance for conducting health risk assessment of chemical mixtures. EPA/630/R-00/002. Washington, DC: Risk Assessment Forum.
- U.S. EPA. 2002a. Guidance on Cumulative Risk Assessment of Pesticide Chemicals That Have a Common Mechanism of Toxicity. Office of Pesticide Programs. At http://www.epa.gov/pesticides/cumulative/methods_tools.htm#guidance
- U.S. EPA. 2002b. The Grouping of a Series of Triazine Pesticides Based on a Common Mechanism of Toxicity. The Office of Pesticide Programs. At: http://www.epa.gov/pesticides/cumulative/common_mech_groups.htm#triazine
- U.S. EPA. 2006. Triazine Cumulative Risk Assessment. The Office of Pesticide Programs. At: http://www.epa.gov/pesticides/cumulative/common_mech_groups.htm#triazine

Mixture Studies from 2008 Ecotox Search

Efficacy Studies

Chadhokar, P. A. and Mani, V. S. Effect of Herbicides on Weed Control and Grain Production in Sorghum.

Int.J.Agric.Sci. 42, 610-613. 72. SOIL, ENV, MIXTURE.

Notes: EcoReference No.: 41360

Chemical of Concern: SZ,ATZ,PPZ,24DXY.

Costa, J. and Appleby, A. P. Response of Two Yellow Nutsedge Varieties to Three Herbicides. Weed Sci. 24(1), 54-

58. 76. ENV,SOIL,MIXTURE. Notes: EcoReference No.: 41686 Chemical of Concern: 24DXY,ATZ.

Dest, W. M., Peters, R. A., and Barrett, M. The Control of Crabgrass and Redroot Pigweed in Silage Corn.

Proc.Northeast.Weed Sci.Soc. 30, 55-58. 76. ENV, SOIL, MIXTURE.

Notes: EcoReference No.: 40803

Chemical of Concern: ATZ,ACR,PDM,24DXY,EPTC,BTY,CZE.

Gaskins, M. H. and Winters, H. F. Herbicide Trials with Young Tropical and Subtropical Fruit and Nut Trees.

Fla.State Hortic.Soc. 75, 418-420. 62. ENV, SOIL, MIXTURE.

Notes: EcoReference No.: 43803 Chemical of Concern: ATZ,DU.

Hammerton, J. L. Weed Control Work in Progress at the University of the West Indies Part 4. PANS

(Pest.Artic.News Summ.) 20(4), 429-436. 74. SOIL, ENV, MIXTURE.

Notes: EcoReference No.: 40607

Chemical of Concern: ATZ,ACR,BT,PDM,CPP,BTY,ODZ,CNR,PMT,TFN,SZ.

Ilnicki, R. D. and Michieka, R. W. Weed Control in Corn with Vel 5052 and Buthiazole (Vel 5026) Applied Alone,

and in Combination with Atrazine and Alachlor. Proc.Northeast. Weed Sci. Soc. 31, 15-19. 77.

ENV,SOIL,MIXTURE.

Notes: EcoReference No.: 41317 Chemical of Concern: ATZ,ACR.

Ilnicki, R. D. and Michieka, R. W. Penoxalin Alone and in Combination with Atrazine, Cyanazine, and Rh 5205 for

Weed Control in Corn. Proc.Northeast.Weed Sci.Soc. 31, 6-10. 77. ENV, SOIL, MIXTURE.

Notes: EcoReference No.: 40870 Chemical of Concern: ATZ,PDM,CZE.

Ivany, J. A. and Sweet, R. D. Sweet Corn Response to Alachlor and Other Herbicides. Proc.Northeast.Weed

Sci.Soc. 25, 47-52, 71. SOIL.ENV.MIXTURE.

Notes: EcoReference No.: 41876

Chemical of Concern: ATZ,SZ,ACR,CZE.

Johnson, B. J. Smutgrass Control with Herbicides in Turfgrasses. Weed Sci. 23, 87-90. 75. SOIL, ENV, MIXTURE.

Notes: EcoReference No.: 40771

Chemical of Concern: BMC,24DXY,ATZ,DU.

Ludwig, J. W. The Use of a Low Dose of Atrazine Alone and in Mixtures with Other Herbicides in the Maize Crop.

Weed Res. 13, 12-18. 73. ENV, SOIL, MIXTURE.

Notes: EcoReference No.: 40618

Chemical of Concern: ATZ,PCH,LNR,24DXY.

Petersen, J. L., Potter, R. L., and Ueckert, D. N. Evaluation of Selected Herbicides for Manipulating Herbaceous

Rangeland Vegetation. Weed Sci. 31, 735-739. 83. ENV, SOIL, MIXTURE.

Notes: EcoReference No.: 41029

Chemical of Concern: 24DXY,ATZ,GYP.

Selleck, G. W. and Sanok, W. J. Herbicides for Weed Control in Sweet Corn and Cabbage. Proc.Northeast.Weed

Sci.Soc. 31, 256-260, 77. ENV.SOIL.MIXTURE.

Notes: EcoReference No.: 40641 Chemical of Concern: ATZ,CZE,ACR.

Smith, D. T., Cooley, A. W., Mooney, D. O., and Wiese, A. F. Herbicidal Control of Volunteer Castorbean. Weed Sci. 20(4), 338-340. 72. SOIL, ENV, MIXTURE.

Notes: EcoReference No.: 41903

Chemical of Concern: MSMA,FMU,PMT,CNT,DMB,DU,ATZ,24DXY,LNR,PPZ.

Tanaka, J. S., Romanowski, R. R. Jr., Sakuoka, R. T., and Crozier, J. A. Jr. Herbicide Evaluation Studies with Sweetcorn (Zea mays L.) in Hawaii. Hawaii Agric. Exp. Stn. Res. Rep. 194, 3-28. 74. SOIL, ENV, MIXTURE.

Notes: EcoReference No.: 40613

Chemical of Concern: SZ,ATZ,DU,LNR,PMT,24DXP,PCH,BTY.

Analytical Methods

Altenburger, R., Bodeker, W., Faust, M., and Grimme, L. H. Evaluation of the Isobologram Method for the

Assessment of Mixtures of Chemicals: Commination Effect Studies with Pesticides in Algal Biotests.

Ecotoxicol.Environ.Saf. 20(1), 98-114. 90. WATER, AQUA, MIXTURE.

Notes: EcoReference No.: 3023

Chemical of Concern: ATZ, AMTL, MBZ, GFS.

Toxicity Data

Douglas, W. S., McIntosh, A., and Clausen, J. C. Toxicity of Sediments Containing Atrazine and Carbofuran to Larvae of the Midge Chironomus tentans. Environ. Toxicol. Chem. 12, 847-853 (OECDG Data File). 93.

WATER, AQUA, MIXTURE. Notes: EcoReference No.: 7284 Chemical of Concern: ATZ.CBF.

Faust, M., Altenburger, R., Boedeker, W., and Grimme, L. H. Additive Effects of Herbicide Combinations on Aquatic Non-Target Organisms. Sci. Total Environ. 134 (Suppl.2), 941-952. 93.

WATER, AQUA, MIXTURE. Notes: EcoReference No.: 4332

Chemical of Concern: 24DXY,ATZ,BT,GYP,SZ,TRL.

Fitzgerald, C. H., Richards, R. F., Selden, C. W., and May, J. T. Three Year Effects of Herbaceous Weed Control in a Sycamore Plantation. Weed Sci. 23(1), 32-35. 75. SOIL, ENV, MIXTURE.

Notes: EcoReference No.: 41357 Chemical of Concern: SZ,ATZ,AMTR.

Hoagland, K. D., Drenner, R. W., Smith, J. D., and Cross, D. R. Freshwater Community Responses to Mixtures of Agricultural Pesticides: Effects of Atrazine and Bifenthrin. Environ. Toxicol. Chem. 12, 627-637 (OECDG

Data File). 93. WATER, AQUA. Notes: EcoReference No.: 6359 Chemical of Concern: ATZ,BFT.

Roberts, S., Vasseur, P., and Dive, D. Combined Effects Between Atrazine, Copper and pH, on Target and Non Target Species. Water Res. 24(4), 485-491. 90. WATER, AOUA, MIXTURE.

Notes: EcoReference No.: 3139 Chemical of Concern: ATZ,CuS.

Roslycky, E. B. Response of Soil Microbiota to Selected Herbicide Treatments. Can.J.Microbiol. 23(4), 426-433. 77. SOIL, ENV, MIXTURE.

Notes: EcoReference No.: 95289

Chemical of Concern: PAQT,LNR,DU,ATZ,SZ.

Sauls, B. T. Pesticide Risk Mitigation of Avian Wildlife in Iowa Agroecosystems. Ph.D.Thesis, Texas Tech Univ.,

Lubbock, TX , 104 p. 99. ENV,MIXTURE.

Notes: EcoReference No.: 93607

Chemical of Concern: LCYT,TFN,LCF,GYP,IZT,24D,QZF,CRM,TBO,MTL,CZE,PDM.

¹ From registrant submitted data to support registration. Compiled by Office of Pesticide Programs Health Effects Division and Registration Division.

ii Atrazine: LD50= 1869 mg/kg; CI= 1485 to 2487 mg/kg.

N/A= Not Applicable